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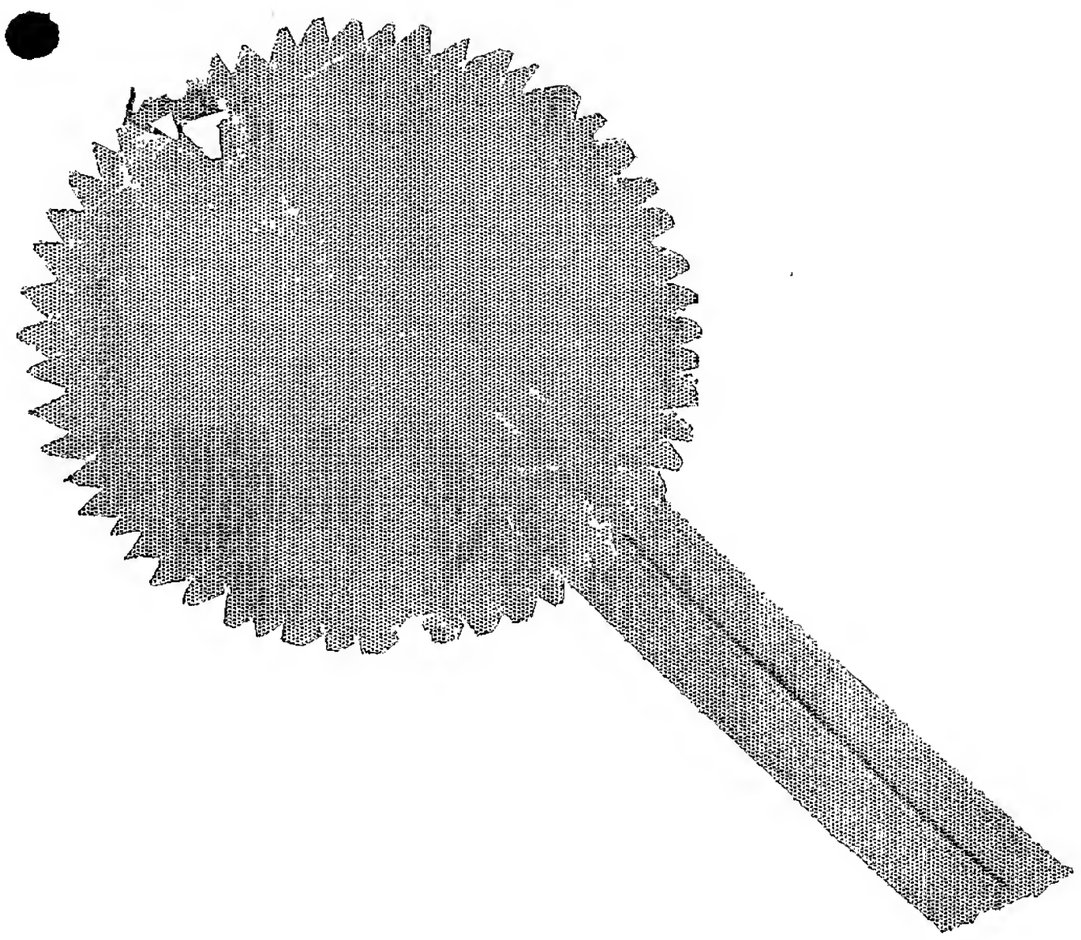
PCT/IN05/00086

सत्यमेव जयते

INTELLECTUAL PROPERTY **INDIA**

GOVERNMENT OF INDIA
MINISTRY OF COMMERCE & INDUSTRY
PATENT OFFICE, DELHI BRANCH
W - 5, WEST PATEL NAGAR
NEW DELHI - 110 008.

I, the undersigned being an officer duly authorized in accordance with the provision of the Patent Act, 1970 hereby certify that annexed hereto is the true copy of the ***Application, Provisional Specification*** *and* ***Drawing Sheets*** *filed in connection with Application for Patent No.* ***501/Del/2004*** *dated* ***18th March 2004.***



Witness my hand this 2nd day of May 2005.

(S.K. PANGASA)
Assistant Controller of Patents & Designs

0501-04

18 MAR 2004

Govt of India Patent Office
New Delhi
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FORM 1

THE PATENT ACT, 1970
(39 of 1970)
APPLICATION FOR GRANT OF A PATENT

BY THE ASSIGNEE AND LEGAL REPRESENTATIVE OF THE TRUE AND FIRST INVENTORS

(See Section 7, 5 (2), 54 and 135, rule 39)

We, **PANACEA BIOTEC LIMITED** of B-1 ,Extn. A/27 Mohan Co-operative, Indl. Estate, Mathura Road, New Delhi – 110044, A Company registered under " The Companies Act 1956.

Hereby declare :-

(i) That we are in possession of an invention for "**NOVEL COMPOSITIONS FOR TOPICAL DELIVERY**".

(ii) That the provisional specification relating to this invention is filed with this application

(iii) That there is no lawful ground of objection to the grant of a patent to us.

(iv) Further declare that the inventors for the said invention are

ORIGINAL

RAJESH JAIN	**DR. KOUR CHAND JINDAL**	**SUKHJEET SINGH**	**DR. VAIBHAV SIHORKAR**
Joint Managing Director	**Executive Vice President – R & D**	**General Manager – R & D**	**Asst. Manager - DDR**
Panacea Biotec Limited.,	Panacea Biotec Limited.,	Panacea Biotec Limited.,	Panacea Biotec Limited.,
B-1 ,Extn. A/27	B-1 ,Extn. A/27	B-1 ,Extn. A/27	B-1 ,Extn. A/27
Mohan Co-operative,	Mohan Co-operative,	Mohan Co-operative,	Mohan Co-operative,
Indl. Estate, Mathura Road,	Indl. Estate, Mathura Road,	Indl. Estate, Mathura Road,	Indl. Estate, Mathura Road,
New Delhi – 110044	New Delhi – 110044	New Delhi – 110044	New Delhi – 110044
INDIAN.	INDIAN.	INDIAN.	INDIAN.

(v) That we are assignee of the true and first inventors.

(vi) That our address for service in India is as follows :

PANACEA BIOTEC LIMITED
B-1 ,Extn. A/27 Mohan Co-operative,
Indl. Estate, Mathura Road,
New Delhi – 110044,.INDIA.

(vii) Following declaration was given by the inventors :

We, the true and first inventors for this invention declare that the applicants herein are our assignee

RAJESH JAIN	DR. KOUR CHAND JINDAL	SUKHJEET SINGH	DR. VAIBHAV SIHORKAR
Joint Managing Director	**Executive Vice President – R & D**	**General Manager – R & D**	**Asst. Manager - DDR**
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B-1 ,Extn. A/27	B-1 ,Extn. A/27	B-1 ,Extn. A/27	B-1 ,Extn. A/27
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New Delhi – 110044	New Delhi – 110044	New Delhi – 110044	New Delhi – 110044
INDIAN.	INDIAN.	INDIAN.	INDIAN.

(viii) That the best of my knowledge, information and belief, the facts and matters stated herein are correct and there is no lawful ground of objection to the grant of patents.

(ix) Following are the attachments with the application.

a) Provisional/complete Specification (3 copies)
b) Power of Authority. (enclosed/not enclosed)
c) Fee Rs. 3000/- by cash/cheque bearing No. dated Drawn on

We request that a patent may be granted to us for the said invention.

Dated this 18th day of March 2004.

For Panacea Biotec Limited.,

Rajesh Jain
Joint Managing Director

TO,
THE CONTROLLER OF PATENTS
THE PATENT OFFICE
NEW DELHI

0501-DEL-04

FORM 2

THE PATENTS ACT, 1970

(39 of 1970)

Provisional Specification

(See Section 10; Rule 13)

ORIGINAL

NOVEL COMPOSITIONS FOR TOPICAL DELIVERY

Panacea Biotec Ltd.

B-1 Extn. A-27. Mohan Co-operative Industrial Estate

Mathura Road

New Delhi – 110 044

The following specification describes the nature of this invention:

FIELD OF THE INVENTION

The present invention relates to pharmaceutical compositions, process of preparation of such compositions, and method for the management of microbial and/or fungal infections of the skin layers. Specifically, the present invention relates to topical compositions comprising of a therapeutic agent(s) either alone or in combination, that are highly effective in the management of microbial and/or fungal infections of the upper skin layers, particularly epidermis and dermis.

BACKGROUND OF THE INVENTION

Terbinafine hydrochloride is a synthetic allylamine derivative. Chemically, Terbinafine hydrochloride is (E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methyl-1-naphthalene-methanamine hydrochloride.

Terbinafine is an approved topical antifungal agent. Terbinafine hydrochloride exerts its antifungal effect by inhibiting squalene epoxidase, a key enzyme in sterol biosynthesis in fungi. This action results in a deficiency in ergosterol and a corresponding accumulation of sterol within the fungal cell.

Terbinafine has been disclosed in U.S. Patent No. 4,755,538, which reports a number of methods for the preparation thereof. Several articles have been published emphasizing the pharmaceutical properties of Terbinafine; see Petranyl, G. et al; Science, 1984, 24, 1239; Stutz. A. et al, J. Med. Chem., 1984, 27, 1539.

U.S. Patent No. 6,383,471 describes compositions and methods for improved delivery of ionizable hydrophobic therapeutic agents. However these compositions do not require a combination of surfactants as an essential feature of the invention. Also there is no indication of gelation of oily components using mixture of surfactants for the topical delivery of drugs.

U.S. Patent No. 6,395,300 discloses method for making a porous matrix of drug by dissolving the drug in a volatile organic solvent to form a drug solution. The method involves combination of at least one volatile pore forming agent with the volatile organic drug solution to form an emulsion, suspension or second solution and removing the volatile organic solvent and volatile pore forming agent from the emulsion, suspension,

or second solution to yield the porous matrix comprising drug. However, this composition is devoid of surfactant architecture and not for topical application.

U.S. Patent Nos. 6,451,339, 6294192 and 6309663 disclose pharmaceutical formulations for administration of hydrophobic lipid-regulating agent, comprising a therapeutically effective amount of the lipid-regulating agent and a carrier, where the carrier is formed from a combination of a hydrophilic surfactant and a hydrophobic surfactant. These compositions use a blend of surfactants; the said compositions upon dilution with aqueous solvent form a clear, aqueous dispersion of the surfactants containing the therapeutic agent. However these compositions neither relate to solvent gelling properties of blends of surfactants nor are they meant for topical use.

U.S. Patent No. 6,455,592 discloses use of penetration agents in dermatological compositions for the treatment of onychomycosis, and corresponding compositions with a pharmaceutically effective amount of Terbinafine hydrochloride, a solvent medium comprising water and at least one straight- or branched-chain C_2–C_8 alkanol and a hydrophilic penetration agent.

U.S. Patent No. 5,446,070 discloses compositions and methods for topical administration of pharmaceutically active agents. However, this is a bio-adhesive composition for topical application and does not essentially contain lipophilic solvents and/or surfactants.

U.S. Patent No. 5,593,680 discloses a cosmetic or dermopharmaceutical compositions in the form of aqueous gels modified by the addition of expanded microspheres.

U.S. Patent Nos. 5,660,839 and 5,939,083 disclose a nongreasy, nonsticky cosmetic/dermatological composition comprising at least one fatty substance and an amount of deformable hollow particulate effective to avoid the greasy and/or sticky feel otherwise attributable to said at least one fatty substance, said deformable hollow particulates comprising a copolymer of vinylidene chloride, acrylonitrile and a (meth) acrylic comonomer.

U.S. Patent No. 5,665,386 discloses use of essential oils to increase bioavailability of oral pharmaceutical compounds that does not disclose usage of a specific blend of surfactants to cause gelation of such oils.

U.S. Patent Nos. 5,681,849 and 5,856,355 disclose topical pharmaceutical compositions comprising of Terbinafine in free base form or in acid addition salt form, water, a lower alkanol, and a water-soluble or water miscible nonionic surfactant, wherein no anionic surfactant is present and wherein said composition is an emulsion gel or lotion, further comprising an oil phase and a thickener. However, this invention does not pertain to the use of surfactant blends for the gelation of the solvents as a carrier for hydrophobic drugs.

None of the literature available in the art discloses compositions that comprise of a therapeutic agent(s) and a blend of surfactants to produce gelation of solvent component(s) containing the therapeutic agent(s) as essential ingredients, which would lead to highly effective topical preparations for extended duration of activity. Hence, there still exists an unmet need to develop highly effective topical compositions for the management of the anti-microbial or anti-fungal infections of the skin which can produce the desired effects for extended periods of time with minimal systemic absorption thus avoiding the undue toxicity of drugs.

OBJECTIVE OF THE INVENTION

It is an objective of the present invention to provide pharmaceutical compositions comprising of a therapeutic agent(s), its salts, esters, hydrates or derivatives; a combination of surfactants; a solvent system; optionally stabilizing agent(s), and other pharmaceutically acceptable excipients, for the management of microbial and/or fungal infections of the skin.

It is another objective of the present invention to provide process for preparation of pharmaceutical compositions comprising of a therapeutic agent(s), its salts, esters, hydrates or derivatives; a combination of surfactants; a solvent system; optionally stabilizing agent(s) and other pharmaceutically acceptable excipients, for the management of microbial and/or fungal infections of the skin.

It is yet another objective of the invention to provide methods of treating a patient using these compositions, which result in an enhanced localization of hydrophobic and/or amphiphilic therapeutic agents for the management of microbial and/or fungal infections of the skin.

It is still another objective of the present invention to provide essentially non-greasy and easily water washable pharmaceutical compositions for topical/dermatological delivery.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides pharmaceutical compositions comprising of a therapeutic agent(s), its salts, esters, hydrates or derivatives; a combination of surfactants; a solvent system; optionally stabilizing agent(s), and other pharmaceutically acceptable excipients.

Specifically, the present invention provides pharmaceutical compositions comprising of terbinafine, its salt, esters, hydrates or derivatives, a combination of surfactants; a solvent system; optionally stabilizing agent(s), and other pharmaceutically acceptable excipients.

In an embodiment of the present invention, the combination of surfactants include lipophilic and hydrophilic surfactants being present in a ratio from 1:5 to 1:20 by weight, preferably 1:10 by weight.

In another embodiment, the composition of the present invention comprises an optimized combination of surfactants as gelators of the solvent system, said surfactant comprising a hydrophilic surfactant component, containing at least one non-ionic hydrophilic surfactant having an HLB value greater than or equal to about 10; and a lipophilic surfactant component, said lipophilic surfactant component being present in an amount sufficient to achieve the required concentration ratio to bring the gelation of the solvent system and is a compound or mixture of compounds having an HLB value less than about 10.

The hydrophilic surfactant of the present invention is selected from but not limited to the group consisting of polyoxyethylene alkyl ethers; polyoxyethylene sorbitan fatty acid esters known as Polysorbates; polyoxyethylene alkyl phenols; polyethylene glycol fatty acid esters; polyoxyethylene-polyoxypropylene block copolymers; polyglycerol fatty acid esters; polyoxyethylene glycerides; polyoxyethylene sterols; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; sugar esters; sugar ethers; sucroglycerides; macrogol glycerides; bile acids and salts, analogues, and derivatives thereof; propylene glycol alginate; lecithins and hydrogenated lecithins; lysolecithin and

hydrogenated lysolecithins; lysophospholipids and derivatives thereof; phospholipids and derivatives thereof; salts of fatty acids; or mixtures thereof.

The lipophilic surfactant of the present invention is selected from but not limited to the group consisting of fatty acids; sorbitan fatty acid esters; acetylated glycerol fatty acid esters; lower alcohol fatty acids esters; trans-esterification products of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, triglycerides and polyalkylene polyols; sterols and sterol derivatives; pentaerythritol fatty acid esters and polyalkylene glycol ethers; monoglycerides and acetylated, e.g. mono-and di-acetylated monoglycerides; or mixtures thereof.

In the present invention, the solvent system is selected from but not limited to the group consisting of oily components, lipophilic solvents, hydrophilic solvents, or mixtures thereof, and optionally may contain aqueous constituents.

The oily components include natural oils, mono-, di-or triglyceride esters of oils selected from a group consisting of medium chain triglycerides, oleic acid, ethyl oleate, ethyl caprylate, ethyl butyrate, isopropyl myristate, croda oil, soybean oil, canola oil or their mono-and di-glycerides, aluminium monostearate, aluminium distearate, aluminium tristearate, microcrystalline wax, petroleum and mixtures, used either alone or in combination thereof.

The lipophilic solvents include triethyl citrate, acetyl butyl citrate or triacetin, triglyceride selected from the group consisting of vegetable oils, fish oils, animal fats, hydrogenated vegetable oils, partially hydrogenated vegetable oils, synthetic triglycerides, modified triglycerides, fractionated triglycerides, and mixtures, used either alone or in combination thereof.

Hydrophilic solvents are selected from but not limited to a group consisting of water, glycols, for example propylene glycol, butylene glycol, hexylene glycol, ethylene glycol and the polyethylene glycols; and mixtures, used either alone or in combination thereof.

In an embodiment of the present invention, the solvent system comprises of at least one oily component(s) and/or at least one lipophilic solvent(s), and optionally hydrophilic solvent(s); the said composition may further contain from 1% to 30% by weight of aqueous phase relative to the total weight of the composition.

In an embodiment, the composition of the present invention optionally contains stabilizing agent(s) selected from a group of naturally occurring polysaccharides which presumably act as structure former and stabilizing agent of oily components in the topical formulations which range from an emulsion, cream, lotion or gel in their consistency and architecture.

The stabilizing agent(s) useful in the present invention are selected from a group of natural polymers and carbohydrates such as chitosan, alginates, carrageenan, cellulose derivatives, pectin, starch, xanthan gum, albumin, alginate, gelatin, acacia, cellulose dextran, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, hyaluronic acid, carboxyethyl cellulose, carboxymethyl cellulose, colloidal silicon dioxide and derivatives thereof.

In an embodiment of the present invention, the other pharmaceutically acceptable excipients are selected from the group comprising of preservatives, formulation aids, antioxidants, diluents, pH adjusting agents, buffering agents, and the like.

In an embodiment of the present invention, the preservative is selected from a group comprising of parabens, benzalkonium chloride, benzyl alcohol, and the like.

In an embodiment of the present invention, the formulation aid is selected from a group comprising of citric acid, tartaric acid, and the like.

In an embodiment, the compositions of the present invention can optionally incorporate sufficient amount of aqueous phase without changing the lipid microenvironment and gel architecture of the composition. The pharmaceutical compositions of the present invention are preferably a gelled topical system with a rich lipid microenvironment, but easily water washable.

In an essential embodiment, the present invention overcomes the problems associated with drug localization in the upper skin layers by providing unique gelator-based lipidic microenvironment.

In an embodiment, the compositions of the present invention are in the form of a cream, gel, jelly, lotion, ointment, topical solution or the like.

In another embodiment, the compositions of the present invention is meant for topical administration for hydrophobic and/or amphiphilic therapeutic agent(s), including but not limited to antibacterial, antifungal, anti-parasite, anti-mycotic, antibiotic, anti-inflammatory, analgesic (narcotic and non-narcotic), anti-septic, disinfectant, anti-psoriatic, anti-eczema, anti-ageing, anti-histaminic, anti-pruritic, keratolytic, anti-seborrheic, gluco-corticoid, muscle relaxant, anti-viral, anesthetic, anti-allergic, or their salts, esters, hydrates or derivatives, used either alone or in combination thereof.

Surprisingly, the present inventors have found that compositions comprising of a combination of a lipophilic and hydrophilic surfactant can gelate a combination of oily and lipophilic solvents (collectively referred to as 'solvent system') and optionally may incorporate sufficient amount of aqueous phase without changing the lipid microenvironment and gel architecture. Use of these compositions result in an enhanced localization of hydrophobic and/or amphiphilic therapeutic agent(s) for the treatment of microbial and/or fungal infections of the skin layers.

In the present invention, the gelator components (combination of surfactants) provide gelation of the solvent system and thus form a three dimensional network. This is due to the fact that surfactant molecules try to self-assemble in solvent environment. The aggregator associate with others through contact points, and thus three-dimensional networks are established, which immobilize the solvent and acts as gel. The addition of aqueous components do not generally break these tubular and torroid structures and furthermore, the stabilizing agent(s) emulsify the excess oil, which has not been gelated during the process of gelation. This also provides a cosmetic appearance to the cream. Further, this highly lipohilic microenvironment on interaction with skin lipids is intended to form a depot within the skin layers through which the entrapped hydrophobic drug could be released over an extended period of time in the localized area where the onychomycosis pathogens generally harbor.

In a preferred embodiment, the therapeutic agent(s) present in the pharmaceutical compositions of the invention are about 0.1% to about 10% by weight, based on the total weight of the pharmaceutical composition.

In a further embodiment, the compositions of the present invention can be in the form of any topical/dermatological dosage for such as creams, gels, ointments, lotions, and the like.

In a further embodiment, the composition of the present invention comprise additional therapeutic agent(s) selected from the group comprising of antibiotic, antibacterial, antifungal, anti-parasite, anti-mycotic, anti-septic, disinfectant, anti-psoriatic, anti-eczema, anti-ageing, anti-inflammatory, analgesic (narcotic and non-narcotic), anti-histaminic, anti-pruritic, keratolytic, anti-seborrheic, gluco-corticoid, muscle relaxant, anti-viral, anesthetic, anti-allergic, and their salts, esters, hydrates or derivatives, used either alone or in combination thereof.

In a still further embodiment, the additional therapeutic agent is an analgesic and/or anti-inflammatory agent selected from but not limited to a group comprising of nimesulide, acetaminophen, acetanilide, acetylsalicylates, acetylsalicylic acid, alminoprofen, aspirin, benoxaprofen, carbamazepine, diflunisal, enfenamic acid, etodolac, fenoprofen, flufenamic acid, flurbiprofen, diclofenac, ibufenac, piroxicam, indomethacin, indoprofen, ketoprofen, ketorolac, miroprofen, morpholine salicylate, naproxen, phenacetin, phenyl salicylate, quinine salicylate, salicylamide, salicylic acid, salicylates and their derivatives, tenoxicam, tolfenamic acid, tramadol etc., or their salts, esters, hydrates or derivatives thereof.

The present invention also provides process for the preparation of pharmaceutical compositions comprising of a therapeutic agent(s), its salts, esters, hydrates or derivatives; a combination of surfactants; a solvent system; optionally stabilizing agent(s) and other pharmaceutically acceptable excipients, for the management of microbial and/or fungal infections of the skin.

In an embodiment, the process of preparation of compositions of the present invention comprises the preparation of an oily phase by mixing the ingredients under temperature and stirring followed by addition of the therapeutic agent(s) with stirring to obtain the desired product.

In another embodiment, the process of preparation of compositions of the present invention comprises the preparation of an oily phase by mixing the ingredients under temperature and stirring followed by addition of the therapeutic agent(s) with stirring; preparation of an aqueous phase by mixing the ingredients under temperature and stirring; adding the said aqueous phase to the said oily phase, under temperature and stirring to obtain the desired product.

The present invention also provides methods for the management of microbial and/or fungal infections of the skin using such pharmaceutical compositions, which provide enhanced localization of the therapeutic agent in the upper skin [illegible] extended period of time.

In order to illustrate embodiments of the present invention, the following examples are provided. However, these examples do not intent to limit the scope of the invention.

EXAMPLES

Example 1

S. No.	*Ingredients*	*Quantity (mg/g)*
1.	Terbinafine hydrochloride	0.010
2.	Sorbitan stearate	0.250
3.	Polysorbate 20	0.025
4.	Medium chain triglyceride	0.250
5.	Isopropyl myristate	0.255
6.	Propylene glycol	0.200
7.	Benzyl alcohol	0.010

The topical formulation was prepared as follows.

Predetermined weighed amounts of Sorbitan stearate, Polysorbate 20, Medium chain triglyceride, Propylene glycol, Isopropyl myristate and Benzyl alcohol were taken. The contents were heated with continuous stirring in a constant temperature water bath while maintaining the temperature of the mass at 60-65°C.

Terbinafine Hydrochloride was added in the melt, while stirring until homogenous mixing was achieved. The off-white to cream-colored formulation thus obtained can be stored in tightly closed HDPE container.

Example 2

S. No.	*Ingredients*	*Quantity (mg/g)*
1.	Terbinafine hydrochloride	0.010
2.	Sorbitan stearate	0.250
3.	Polysorbate 20	0.025
4.	Medium chain triglyceride	0.250

5.	Propylene glycol	0.075
6.	Chitosan	0.040
7.	Citric acid	0.090
8.	Benzyl alcohol	0.010
9.	Purified water	0.250

The topical formulation was prepared as follows.

An oily phase was prepared first. Predetermined weighed amounts of Sorbitan stearate, Polysorbate 20, Medium chain triglyceride, Propylene glycol and Benzyl alcohol are taken; the liquid ingredients were passed through nylon cloth and transferred to a jacketed S.S. container. The solid ingredients were added to the contents of the S.S. container and mixed. This mixture was heated with continuous stirring by circulating hot water in the jacket while maintaining the temperature of the mass at 60-65°C. Terbinafine hydrochloride was added in the above melt, while stirring until homogenous mixing was achieved.

An aqueous phase was then prepared. Predetermined weighed amounts of Chitosan and Citric acid were mixed with sufficient purified water and the mixture was heated with continuous stirring while maintaining the temperature of the mass at 60-65°C.

The oily phase and aqueous phase were maintained at 60-65°C and bulk of aqueous phase was added to oily phase maintaining the similar temperature (60—65°C) with continuous stirring to obtain the desired formulation.

Example 3

S. No.	*Ingredients*	*Quantity (mg/g)*
1.	Terbinafine Hydrochloride	0.010
2.	Nimesulide	0.010
2.	Glyceryl monostearate	0.250
3.	Polysorbate 20	0.050
4.	Propylene glycol	0.320
5.	Isopropyl myristate	0.350
6.	Benzyl alcohol	0.010

The topical formulation was prepared as follows.

Predetermined weighed amounts of Glyceryl monostearate, Polysorbate 20, Isopropyl myristate, Propylene glycol and Benzyl alcohol were taken. The contents were heated with continuous stirring while maintaining the temperature of the mass at 60-65°C. Terbinafine hydrochloride and Nimesulide were added in melt, while stirring until homogenous mixing was achieved. The off-white to cream-colored formulation thus obtained was stored in tightly closed HDPE container.

Example 4

S. No.	*Ingredients*	*Quantity (mg/g)*
1.	Clotrimazole	0.010
2.	Polyethylene glycol distearate	0.250
3.	Polysorbate 20	0.025
4.	Mineral oil	0.250
5.	Chitosan	0.040
6.	Citric acid	0.080
.7.	Benzyl alcohol	0.010
8.	Purified water	0.335

The topical formulation was prepared as follows.

An oily phase was prepared first. Predetermined weighed amounts of Polyethylene glycol distearate, Polysorbate 20, Medium chain triglyceride, Mineral oil and Benzyl alcohol were taken; the liquid ingredients were passed through nylon cloth and transferred it to a jacketed S.S. container. The solid ingredients were added to the contents of the S.S. container and mixed. This mixture was heated with continuous stirring by circulating hot water in the jacket while maintaining the temperature of the mass at 60-65°C. Clotrimazole was added in the above melt, while stirring until homogenous mixing was achieved.

An aqueous phase was then prepared. Predetermined weighed amounts of Chitosan and Citric acid were mixed with sufficient purified water and the mixture was heated with continuous stirring while maintaining the temperature of the mass at 60-65°C.

The oily phase and aqueous phase were maintained at 60-65°C and bulk of aqueous phase was added to oily phase maintaining the similar temperature (60—65°C) with continuous stirring to obtain the desired product.

Example 5

S. No.	*Ingredients*	*Quantity (mg/g)*
1.	Miconazole	0.020
2.	Gentamycin sulphate	0.010
3.	Polyethylene glycol distearate	0.250
4.	Polysorbate 20	0.025
5.	Isopropyl myristate	0.250
6.	Chitosan	0.040
7.	Citric Acid	0.080
8.	Benzyl alcohol	0.010
9.	Purified Water	0.315

The topical formulation was prepared as follows.

An oily phase was prepared first. Predetermined weighed amounts of Polyethylene glycol distearate, Polysorbate 20, Isopropyl myristate and Benzyl alcohol were taken; the liquid were passed ingredients through nylon cloth and transferred to a jacketed S.S. container. The solid ingredients were added to the contents of the S.S. container and mixed. This mixture was heated with continuous stirring by circulating hot water in the jacket while maintaining the temperature of the mass at 60-65°C. Miconazole and Gentamycin sulphate were added in the above melt, while stirring until homogenous mixing was achieved.

An aqueous phase was prepared. Predetermined weighed amounts of Chitosan and Citric acid were mixed with sufficient purified water and the mixture was heated with continuous stirring while maintaining the temperature of the mass at 60-65°C. The oily phase and aqueous phase were maintained at 60-65°C and bulk of aqueous phase was added to oily phase maintaining the similar temperature (60—65°C) with continuous stirring to obtain the desired product.

Example 6

S. No.	*Ingredients*	*Quantity (mg/g)*
1.	Sertaconazole	0.010
2.	Sorbitan stearate	0.250
3.	Polysorbate 20	0.025
4.	Medium chain triglyceride	0.250
5.	Propylene glycol	0.075
6.	Chitosan	0.040
7.	Citric acid	0.090
8.	Benzyl alcohol	0.010
9.	Purified water	0.250

The topical formulation was prepared as follows.

An oily phase was prepared first. Predetermined weighed amounts of Sorbitan stearate, Polysorbate 20, Medium chain triglyceride, Propylene glycol and Benzyl alcohol are taken; the liquid ingredients were passed through nylon cloth and transferred to a jacketed S.S. container. The solid ingredients were added to the contents of the S.S. container and mixed. This mixture was heated with continuous stirring by circulating hot water in the jacket while maintaining the temperature of the mass at 60-65°C. Sertaconazole was added in the above melt, while stirring until homogenous mixing was achieved.

An aqueous phase was then prepared. Predetermined weighed amounts of Chitosan and Citric acid were mixed with sufficient purified water and the mixture was heated with continuous stirring while maintaining the temperature of the mass at 60-65°C.

The oily phase and aqueous phase were maintained at 60-65°C and bulk of aqueous phase was added to oily phase maintaining the similar temperature (60—65°C) with continuous stirring to obtain the desired formulation.

DERMATOPHARMACOKINEIC STUDY

The Dermatopharmacokinetic (DPK) studies in the present invention are used to mimic clinical trials as a means of documenting bioavailability and equivalence of topical drug products. For the therapeutic class of anti-fungal drugs, the stratum comeum itself is the site of action. For example, in fungal infections of the skin, fungi reside in the stratum

comeum and therefore DPK measurement of an antifungal drug in the stratum comeum represents direct measurement of drug concentration at the site of action. No better assay of bioequivalence can be envisioned for this class of compounds than direct assay of the target tissue.

The "Tape stripping" method used is capable of demonstrating differences of stratum corneum (SC) localization of the said invention over competitor products. This is determined by applying different compositions of the said invention to the skin surface for a specified exposure time, adhesive films are put on the treated skin and are taken off again after a certain application time and analysis of the localized amount in stratum corneum using validated analytical method to measure the localization index in the stratum corneum per unit surface of applied area.

Dermatopharmacokinetic (DPK) study was done to determine the comparative efficacy of Terbinafine HCl topical formulations of Innovator product (Lamisil®, herein referred to as INV) and Panacea Biotec Ltd. (DDR-FRD-F1, herein referred to as PBL). The composition described in Example 2 above has been coded as DDR-FRD-F1 and used for the said study.

The assay values of the compositions used for the study were as follows: Lamisil® contained 0.099 mg of Terbinafine HCl per mg of cream formulation and DDR-FRD-F1 (coded for Example 2) contained 0.090 mg of Terbinafine HCl per mg of cream formulation.

Tape stripping experiment is performed following the drafted guidance of US FDA *(Guidance for Industry: Topical dermatological drug product NDAs and ANDAs- In vitro bioavailability, bioequivalence, in vitro release and associated studies)*. The general test procedure in the mentioned study is summarized below:

First, the hair of the experimental animal (guinea pig) is removed by plucking (preferably) and then the animals are exposed in a conditioned room preferably at 20°C with 60% RH. This condition has to be maintained throughout the experimental period. The dorsal side of the guinea pigs (2x2.5 cm^2) is marked preferably at two sites on dorsa. Control is run simultaneously to check baseline reading.

About 65.0 to 125.0 mg of the formulations (1% topical creams i.e. 100.0 mg formulations contain 1 mg of active drug, Terbinafine hydrochloride) were applied to the stratum corneum of 5 guinea pigs (N=5 denoted as N1, N2, N3, N4 and N5). A non-occluding protective guard is placed to cover the application area (non-occluding aluminum foil is used). The excess formulation is removed after 15 minutes from the application site by wiping three times lightly with a cotton swab. The initial and final weight of the cotton swab is measured to precisely monitor applied amount per square meter of the skin.

After appropriate time intervals, the samples are collected following tape stripping using adhesive tape. Transpore™ tape (Model 1527-1, surface area 2.5 cm^2, 3M) is used as an adhesive tape. The adhesive tape is applied using uniform pressure and removed at different time intervals using constant peel off force. The duration of the study was 24 hours at the following intervals: 0.5, 1.0, 3.0, 6.0, 12.0 and 24.0 hours. A blunt ended forceps is used to apply individual adhesive tap with a constant pressure, by the same investigator every time. Both test and reference products are applied on the same side to counterbalance the inter-subject variation.

The procedure is repeated for each site at designated time points. The drug is extracted from the combined eight tape stripping and the concentration is determined using a validated analytical method. The first two tape strips are removed and not included in the analytical method validation (to accommodate residual product contamination). Further 8 tape strips are taken and pooled for each time interval and analyzed using validated method of estimation for Terbinafine hydrochloride. Tape stripping samples are stored in 10 ml polypropylene tube with 7.0 ml of 80:20 v/v acetonitrile and TEA (0.72 mM) at pH 2.5 and subjected to agitation for 16 h. In case of delay, samples are stored at -70°C until processed. Supernatant is passed through 0. 45 µm filter and subjected to validated analytical HPLC method. The results of the study are expressed as concentration of drug (nmoles) calculated to be in stratum corneum (SC) per cm^2 of the applied area (i.e. calculation for 100 nmol per cm^2 of applied cream). The results of the study are presented in tables 1-3 and in figure 1, as mentioned below.

Table 1: Calculation for drug localization of Inventor's formulation (PBL) in stratum corneum

Table 2: Calculation for drug localization of Innovator's formulation (INV) in stratum corneum

Table 3: Comparative efficacy of Inventor's formulation (PBL) over Innovator's formulation (INV) from Dermatopharmacokinetic (DPK) studies

Figure 1: Comparative Dermatopharmacokinetic (DPK) profile of the Inventor's formulation (PBL) and Innovator's (INV) formulation

The results of dermatopharmacokinetic study showed a significant increase in localization of Terbinafine HCl on the skin (stratum corneum), and hence improved efficacy of the composition of the present invention over the Innovator product (Lamisil®).

Table 1: Calculation for drug localization of Inventor's formulation (PBL) in stratum corneum

Application codes	nmoles/cm^2 (drug) applied	nmoles/cm^2 (drug) In SC	Equilibrated nmoles/cm^2 drug in SC per 100 nmoles/cm^2 drug applied
PBL-0.5-N1	551.93	38.44	6.964
PBL-0.5-N2	485.61	69.55	14.32
PBL-0.5-N3	419.84	30.43	7.247
PBL-0.5 N4	564.84	116.20	20.57
PBL-0.5 N5	421.26	39.75	9.435
PBL-1.0-N1	543.53	19.73	3.629
PBL-1.0-N2	549.19	15.56	2.833
PBL-1.0-N3	565.63	75.78	13.39
PBL-1.0-N4	555.61	49.04	8.830
PBL-1.0-N5	604.48	39.53	6.539
PBL-3.0-N1	307.59	48.75	15.84
PBL-3.0-N2	429.92	67.82	15.77
PBL-3.0-N3	585.37	21.15	3.613
PBL-3.0-N4	512.79	62.49	12.18
PBL-3.0-N5	457.09	53.5	11.70
PBL-6.0-N1	593.48	20.07	3.381
PBL-6.0-N2	533.52	22.17	4.155
PBL-6.0-N3	665.66	20.49	3.078
PBL-6.0-N4	446.62	57.19	12.80
PBL-6.0-N5	474.19	41.10	8.667
PBL-12.0-N1	590.31	23.76	4.025
PBL-12.0-N2	721.84	33.06	4.579
PBL-12.0-N3	624.28	22.22	3.559
PBL-12.0-N4	501.76	35.18	7.011
PBL-12.0-N5	523.0	13.23	2.530
PBL-24.0-N1	534.39	13.41	2.509
PBL-24.0-N2	380.38	25.17	6.617
PBL-24.0-N3	556.32	19.39	3.485
PBL-24.0-N4	464.26	18.62	4.010
PBL-24.0-N5	420.67	11.15	2.650

Table 2: Calculation for drug localization of Innovator's formulation (INV) in stratum corneum

Application codes	nmoles/cm^2 (drug) applied	nmoles/cm^2 (drug) In SC	Equilibrated nmoles/cm^2 drug in SC per 100 nmoles/cm^2 drug applied
INV-0.5-N1	807.89	47.12	5.832
INV-0.5-N2	783.78	57.72	7.364
INV-0.5-N3	657.11	50.04	7.615
INV-0.5 N4	557.32	100.8	8.010
INV-0.5 N5	537.21	35.15	6.540
INV-1.0-N1	752.41	50.04	6.650
INV-1.0-N2	810.88	34.37	4.238
INV-1.0-N3	824.15	43.17	5.238
INV-1.0-N4	609.39	56.93	9.340
INV-1.0-N5	619.45	49.98	8.060
INV-3.0-N1	728.91	101.41	13.91
INV-3.0-N2	756.01	64.84	8.576
INV-3.0-N3	760.82	46.51	6.113
INV-3.0-N4	455.56	28.39	6.230
INV-3.0-N5	538.98	35.46	6.570
INV-6.0-N1	625.56	25.97	4.151
INV-6.0-N2	775.94	37.30	4.807
INV-6.0-N3	680.68	20.58	3.023
INV-6.0-N4	604.08	42.56	7.040
INV-6.0-N5	514.73	24.05	4.670
INV-12.0-N1	757.85	39.03	5.150
INV-12.0-N2	723.49	25.62	3.541
INV-12.0-N3	745.19	5.88	0.789
INV-12.0-N4	731.38	29.21	3.990
INV-12.0-N5	555.28	18.19	3.270
INV-24.0-N1	756.04	15.07	1.993
INV-24.0-N2	643.9	14.09	2.188
INV-24.0-N3	749.41	2.22	0.296
INV-24.0-N4	792.19	17.09	2.150
INV-24.0-N5	693.40	12.94	1.860

Table 3: Comparative efficacy of Inventor's formulation (PBL) over Innovator's formulation (INV) from Dermatopharmacokinetic (DPK) studies

Application codes	SC localization (nmoles/cm^2) after 100.0 nmoles/cm^2 applied dose				
	N1*	N2*	N3*	N4*	N5*
INNOVATOR'S PRODUCT (LAMISIL^{TM})					
INV-0.5	5.832	7.364	7.615	18.02	6.541
INV-1.0	6.650	4.238	5.238	9.341	8.064
INV-3.0	13.912	8.576	6.113	6.233	6.573
INV-6.0	4.151	4.807	3.023	7.042	4.672
INV-12.0	5.150	3.541	0.789	3.993	3.272
INV-24.0	1.993	2.188	0.296	2.152	1.861
PBL'S PRODUCT (DDR-FRD-F1)					
PBL-0.5	6.964	14.322	7.247	20.571	9.435
PBL-1.0	3.629	2.833	13.397	8.8307	6.539
PBL-3.0	15.849	15.775	3.613	12.181	11.701
PBL-6.0	3.381	4.155	3.078	12.802	8.667
PBL-12.0	4.025	4.579	3.559	7.0115	2.531
PBL-24.0	2.509	6.617	3.485	4.011	2.653

*Number of animals (N=5, guinea pigs) used in the study denoted as N1, N2, N3, N4 and N5

0.5, 1.0, 3.0, 6.0, 12.0 & 24.0 denote time intervals in hours.

Dated this day of March, 2004

For Panacea Biotec Limited.,

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PANACEA BIOTEC LTD.

APPLICATION NO.

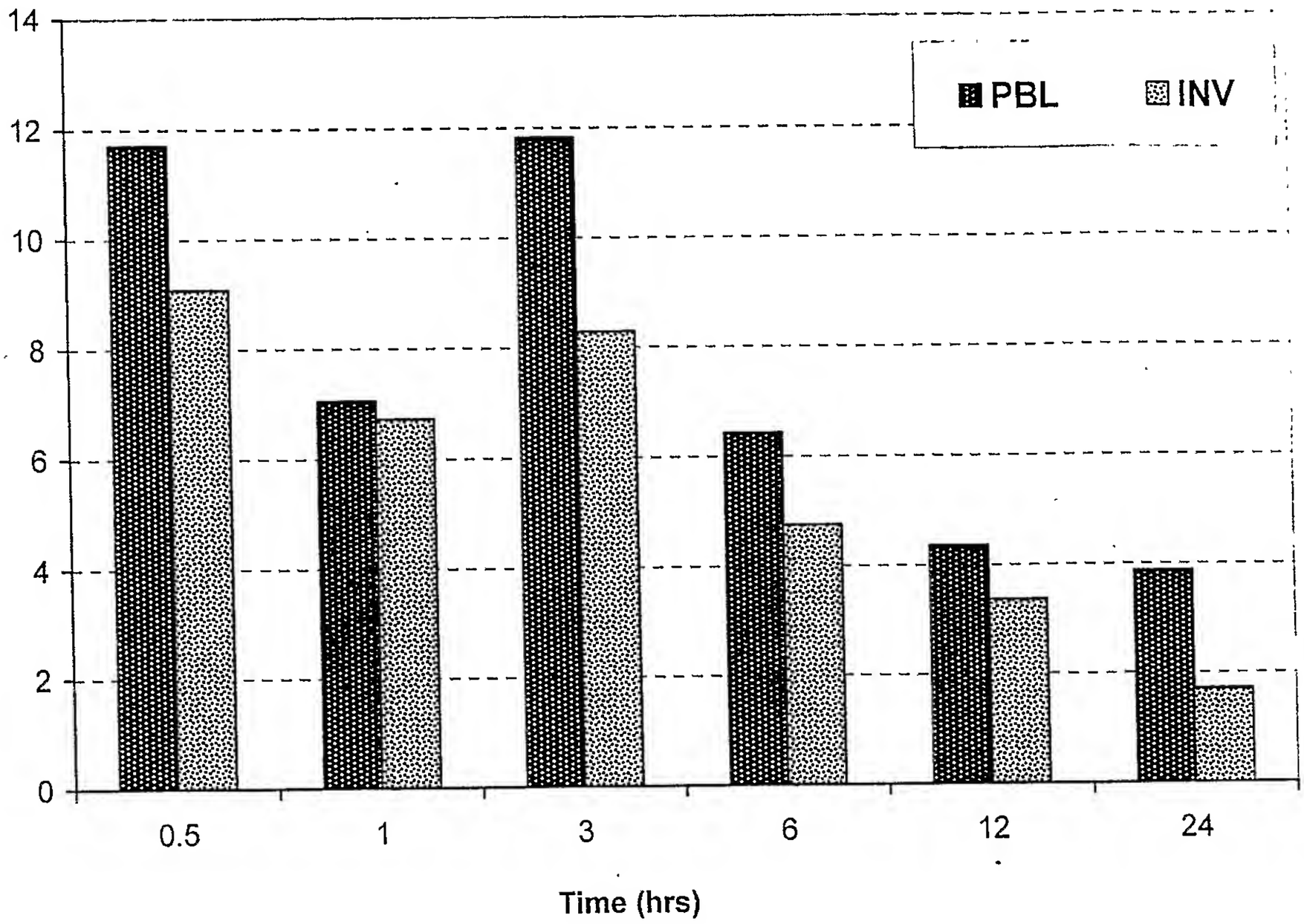
NO. OF SHEETS - 1

SHEET NO. 1

0 6 0 - 0 4

18 MAR 2004

Figure 1: Comparative Dermatopharmacokinetic (DPK) profile of the Inventor's formulation (PBL) and Innovator's (INV) formulation



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